

MARKED-UP CLAIMS

5. A method for detecting a target molecule comprising the steps of:

i) contacting a sample with a locator probe comprising a binding moiety specific for said target molecule and an amplification nucleic acid sequence to produce a target molecule-locator probe complex, said amplification nucleic acid sequence having one or more restriction sites for a restriction endonuclease when hybridised to a complementary strand;

ii) producing an amplification structure bound to any complex produced in the preceding step by performing one or more times the amplification step of treating said sample and locator probe with:

a) a single stranded amplification template comprising:

i) arranged in a 5' to 3' direction:

a) an extension nucleic acid sequence;

b) a hybridisation nucleic acid sequence complementary to the amplification nucleic acid sequence of the previous amplification step or, where there is no previous amplification step, of the preceding step and having substantially the same sequence as said extension nucleic acid sequence; and

c) an amplification moiety, being limited in all but the final amplification step to a nucleic acid sequence; and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence;

b) a polymerising agent capable of extending the 3' terminus of the amplification nucleic acid sequence of the previous amplification step or, where there is no previous amplification step, of the preceding step by synthesising a complementary strand to said extension nucleic acid sequence of said amplification template;

c) said restriction endonuclease; and

d) the reagents and conditions necessary to:

i) effect the action of said polymerising agent and separating agent to allow the extension of the 3' terminus of the amplification nucleic acid sequence of the previous amplification step or, where there is no previous amplification step, of the preceding step by the synthesis of a plurality of sequences complementary to said extension nucleic acid sequence of said amplification template; and

ii) effect dissociation of **fragments of** nucleic acid strands which have been cut by said restriction endonuclease activity from uncut complementary strands whilst not effecting dissociation of uncut nucleic acid strands from uncut complementary strands;

iii) detecting any bound amplification template from the amplification step or steps; and

iv) correlating the results of detection step (iii) with the presence of said target molecule.

8. A method according to claim[s] 5[-7], being performed isothermally.

9. A method according to claim[s] 5[-7], being performed at more than one temperature.

10. A method according to [any one of the preceding claims] **claim 1**, the amplification step of step (ii) being performed two or more times.

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13. A method for detecting a target molecule according to [either one of claims 11 or 12] claim 11, the removal of said amplification template being achieved by the use of a 5' double strand specific exonuclease.

14. A method for detecting a target molecule according to [either one of claims 11 or 12] claim 11, the removal of said amplification template being achieved through the use of elevated temperature.

16. A method for detecting a target molecule according to [any one of claims 1-9] claim 1, prior to said detection step additionally comprising performing a method according to steps (ii) and (iii) of [either one of claims 11 or 12] claim 11.

17. A method for detecting a target molecule according to [any one of claims 1-9] claim 1, said amplification moiety of said amplification template from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing steps (ii)-(iii) of a method according to [either one of claims 11 or 12] claim 11.

18. A method for detecting a target molecule according to [any one of claims 11-15] claim 11, prior to said detection step additionally comprising performing step (ii) of a method according to [any one of claims 1, 3 or 5] claim 1.

19. A method for detecting a target molecule according to [any one of claims 11-15] claim 11, said amplification moiety of said [amplification template] locator probe or additional locator probe from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing step (ii) of a method according to [any one of claims 1, 3 or 5] claim 1.

20. A method for detecting a target molecule according to [any one of claims 1-9, 18 or 19] claim 1, the step of detecting any bound amplification template comprising the steps of:

- i) treating said sample, locator probe and amplification template or amplification templates with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and
- ii) detecting any bound detection probe.

21. A method for detecting a target molecule according to [any one of claims 11-17] claim 12, the step of detecting any bound amplification template comprising the steps of:

- i) treating said sample, locator probe and amplification template with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and
- ii) detecting any bound detection probe.

22. A method according to [either one of claims 20 or 21] claim 20, the detection probe having a label which is detected by any one of the group of luminometry, fluorometry, spectrophotometry, and radiometry.

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24. A method according to [any one of the preceding claims] **claim 1**, the amplification step being performed two or more times, each amplification step being performed using an amplification template having a different extension nucleic acid sequence, hybridisation nucleic acid sequence and amplification moiety to that of the amplification template used in the previous amplification step.
25. A method according to [any one of the preceding claims] **claim 1**, the target molecule to be detected being a nucleic acid sequence and the binding moiety of said locator probe comprising a nucleic acid sequence complementary to said target molecule nucleic acid sequence.
26. A method according to [any one of the preceding claims] **claim 1**, being performed using more than one locator probe, each locator probe having the same amplification nucleic acid sequence.
27. A method according to [any one of the preceding claims] **claim 1**, comprising two repeats.
28. A method according to [any one of the preceding claims] **claim 1**, unreacted reagents being removed at the end of step (i), each repeat, or detection step by washing.

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